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PK-M2 Makes Cells Sweeter on HIF1

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The transcription factor hypoxia-inducible factor 1 (HIF1) facilitates the induction of enzymes necessary for anaerobic glycolysis. Luo et al. (2011) now identify pyruvate kinase (PK)-M2 as an intriguing new interacting partner for HIF1, revealing a potential mechanism for the Warburg effect, an elevation in aerobic glycolytic metabolism frequently observed in cancer.

Cells exposed to low oxygen (hypoxia) undergo a number of phenotypic changes in order to survive, including a shift toward glycolysis, the oxygen-independent mechanism of producing ATP. A major factor driving increased glycolytic flux in hypoxic cells is induction of the transcription factor hypoxia-inducible factor 1 (HIF1), which consists of a stably expressed β subunit and an oxygen-labile α subunit (Wang et al., 1995). Although the oxygen-dependent hydroxylation, ubiquitylation, and degradation of the α subunit provide the primary means of regulating HIF1 activity, other HIF1 modulators have been described in recent years, complicating the picture. In this issue, Luo et al. (2011) reveal a new aspect to this story, showing that the glycolytic isozyme pyruvate kinase-M2 can act as a coactivator for HIF1, greatly increasing the transcriptional activity of HIF1.

Pyruvate kinase (PK) is the final enzyme in glycolysis, converting phosphoenolpyruvate to pyruvate with the production of ATP. There are four forms of PK derived from two genes: PK-L and PK-R from *PKLR* and PK-M1 and PK-M2 from *PKM2*. Each isozyme is subject to different allosteric, substrate, and posttranslational regulation, making it one of the most highly regulated enzymes in the glycolytic pathway. The PK-M isoforms are produced by alternative splicing such that exon 9 is present in M1 and exon 10 in M2 (Mazurek, 2010). Importantly, PK-M1 is in a permanent "on" state with rapid substrate turnover and ATP production, whereas PK-M2 can switch between low- and high-activity states, depending on the needs of the cell. They are therefore expressed in different cell types: M1 is present in a number of tissue types, including muscle and brain, and M2 in those with high anabolic requirements, such as proliferating cells (including all cells during embryogenesis). In tumors, the predominant form of PK expressed is the M2 variant (Christofk et al., 2008; Reinacher and Eigenbrodt, 1981). This isozyme selection allows for the rapid proliferation observed in tumors but, paradoxically, may not always be consistent with the high lactate production also observed.

The hydroxylation of HIF1α is mediated by a family of prolyl hydroxylase domain (PHD) enzymes (Kaelin and Ratcliffe, 2008). Of the three members (PHD1–3), PHD2 is thought to be the major hydroxylase that is responsible for HIF1α stability. Few targets have been described for the PHDs, which is in part due to the technical challenge of expressing fully active recombinant PHDs and definitively demonstrating changes in hydroxylation state of putative target proteins. The use of mass spectrometry (MS) is currently the gold standard for directly demonstrating the hydroxylation of proline residues in a peptide. It is often, however, not possible to specifically assign the mass change observed by MS to a specific proline residue due to the oxidation of other residues that are present in the peptide. Although this has hampered investigations into PHD substrates thus far, there is no doubt that there are more targets for these enzymes to be discovered.

In the current work, Luo et al. demonstrate that PHD3-mediated hydroxylation of PK-M2 increases the DNA binding of HIF1α and the coactivator p300 and that this leads to increased expression of HIF1 target genes (Figure 1). They describe a new hypoxia response element (HRE) in intron 1 of *PKM2*, showing that this gene is also a target of HIF1. As the

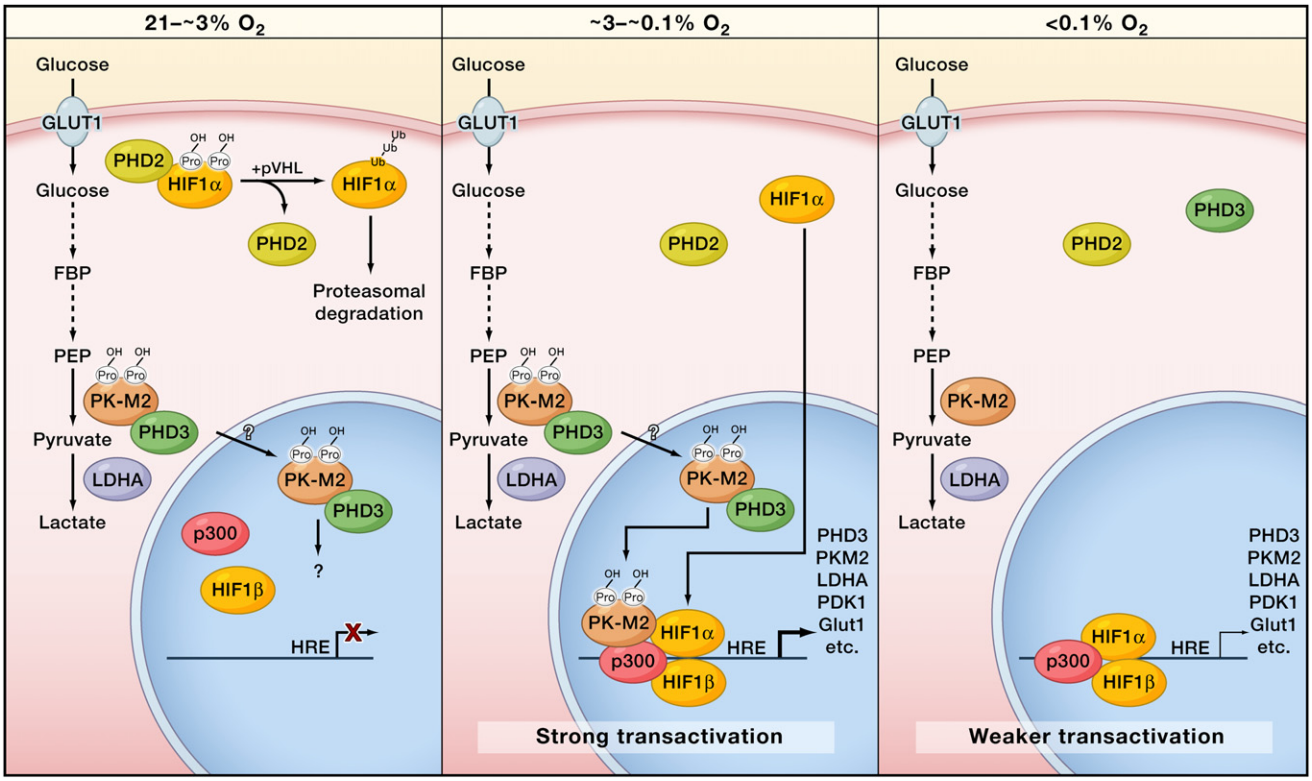

Figure 1. Differential Regulation of HIF1α and PK-M2 by PHDs

As oxygen levels decrease, the transcription factor hypoxia-inducible factor 1 α (HIF1α) is stabilized and can strongly induce its target genes. Once levels are close to anoxia, pyruvate kinase M2 (PK-M2) hydroxylation is decreased, and HIF1 transactivation activity is weaker. FBP, fructose 1,6-bisphosphate; GLUT1, glucose transporter 1; HRE, hypoxia response element; LDHA, lactate dehydrogenase A; PEP, phosphoenol pyruvate; PDK1, pyruvate dehydrogenase kinase 1; PK-M2, pyruvate kinase M2; PHD, prolyl hydroxylase domain; Ub, ubiquitin; pVHL, von Hippel Lindau protein.

authors point out, this could bring about a feedforward mechanism between these three proteins, as *EGLN3* (the gene encoding PHD3) is also a HIF1 target (Marxsen et al., 2004).

Interestingly, HIF1 does not appear to be the only nuclear target of PK-M2. Not only do the authors also show that PK-M2 can also bind and enhance the transactivation of HIF2, but a previous study found that PK-M2 binds the developmental transcription factor Oct4, resulting in increased target gene expression (Lee et al., 2008). However, Luo et al. go further here, showing that the PK-M2:HIF1α interaction is mediated via exon 10, the specific region of PK-M2 not present in the M1 isozyme. When the amino acid sequence of this exon was examined, they found that it contains an LXXLAP motif—the sequence in HIF1α responsible for its hydroxylation by PHD2—and that two proline residues in this exon are hydroxylated. When these residues are mutated, the interaction between HIF1α and PK-M2 is lost. The authors therefore propose an important new mechanistic link between oxygen sensing and the modulation of HIF1 transactivation activity by implicating PHD3 in the hydroxylation of PK-M2. Their data strongly suggest that PHD3-dependent hydroxylation of PK-M2 allows it to bind HIF1α and increase its binding p300 and the occupancy of HIF1 at target gene promoter regions. Although the authors observed a PK-M2/p300 interaction by coimmunoprecipitation, there remains the possibility that this interaction may be indirect (through HIF1, for example).

Luo et al. present data showing that PK-M2 hydroxylation appears to be unaffected by 1% O_2, when you might expect PHD3 to be inactive (as observed with the PHD2-mediated hydroxylation of HIF1). Indeed, near-anorexic conditions are required to reduce PK-M2 hydroxylation at all. This suggests that PHD3 is still very much active in hypoxia, indicating that it has a lower K_M for oxygen than PHD2. Although not in agreement with data from in vitro studies, this is not unlikely. Finally, and importantly, the authors show that depletion of either PHD3 or PK-M2 reduces the transcription of HIF1 metabolic target genes, providing the link between PHD3, PK-M2, and HIF1-mediated glycolytic control.

These data are of particular relevance to cancer, where PK-M2 is thought to be preferentially expressed. As HIF1 can be stabilized in conditions in which oxygen levels are not limiting, this phenomenon is not necessarily limited to hypoxic cells. Glycolytic gene expression could therefore be enhanced in normal oxygen conditions, perhaps leading to the well-known but little understood aerobic glycolysis phenotype observed in tumors almost 100 years ago, known as the Warburg effect (Tennant et al., 2009). The increased glycolytic enzyme expression

downstream of PK-M2 may well resolve the paradox of PK-M2 expression in cells with apparent aerobic glycolysis, but it is unlikely that this is the whole story. Indeed, in a recent paper, Chen et al. show that PHD3 interaction with PK-M2 in the cytosol modulates PK activity, suggesting further complexity within this story (Chen et al., 2011). The re-expression of PK-M2 in tumors appears therefore to have two distinct roles: the alteration of gene expression as well as its well-described metabolic role. However, in light of its binding to Oct4, HIF1, and HIF2, it will be interesting to see how extensive the role of PK-M2 in the nucleus turns out to be.

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